

STZHFD-Induced Type 2 Diabetes Preferentially Upregulates Pro-Inflammatory Chemokines in the Murine Intervertebral Disc When Compared to db/db Mice

Christian Gonzalez¹, Rachana Vaidya¹, Sade Clayton¹, Simon Tang¹

¹Washington University in St. Louis, St. Louis, MO

christian.gonzalez@wustl.edu

Disclosures: None

INTRODUCTION: The intervertebral disc (IVD) degeneration is a strong contributor to the low back pain epidemic. Disc degeneration is characterized by changes in structure, function, and biology of the disc [1, 2], and this degeneration can be exacerbated by chronic diseases including Type 2 Diabetes (T2D). T2D is defined by systemic hyperglycemia and chronic systemic inflammation, which in turn severely affects the musculoskeletal system [3,4]. We have previously shown that T2D promotes IVD-specific-production of inflammatory cytokines in an organ culture model of leptin-receptor-deficient *Lepr^{db}* (db/db) mice [5]. However, because leptin is a pleiotropic adipokine regulating matrix synthesis, hormone signaling, and cytokine production [6, 7], the absence of leptin signaling may attenuate the key inflammatory features crucial for understanding the multi-system crosstalk in low back pain development. We hypothesized that a nongenetic model of T2D may be better recapitulate the physiology of the disease without the potential off target effects of the db/db model for understanding the impact of diabetes on IVD degeneration. In this study, we characterize the disc-specific cytokine production in the Streptozotocin High Fat Diet (STZHFD) murine model of T2D and contrast these findings with our previous work on db/db mice.

METHODS: To induce Type 2 Diabetes, 3-month-old C57BL/6 male mice were fed a high fat diet (HFD; 60% fat kcal; D12492) for 12 weeks, and a single dose of 100mg/kg Streptozotocin was delivered IP after the first 4 weeks of HFD [8]. Control animals were fed standard mouse chow and injected with Na-Citrate buffer. Diabetic status was confirmed via HbA1c (> 6.5%) measured at 8 weeks post injection and oral glucose tolerance test (>50% peak glucose 2 hours post bolus) measured at 12 weeks [9]. After the 12-week HFD regimen and confirmation of the diabetic status, 3 coccygeal function spine units (FSUs) including IVDs were harvested from 9 diabetic and 3 control mice. The FSUs were cultured in 2 mL Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12 Ham with L-glutamine and 15 mM HEPES). Cultures underwent a preconditioning period of 7 days to account for inflammatory response and endogenous degeneration due to harvesting. A multiplex assay of remodeling factors and cytokines (45-Plex Mouse Cytokine Discovery Assay, Eve Technologies Assays; CCL-2,3,4,5,11,12,17,20,21,22; CSF-1,2,3; IL-1 α ,1 β ,2,3,4,5,6,7,9,10,11,12A,12B,13,15,16,17; CXCL1,2,5,9,10; IFN- γ , β 1; TNF α ; LIF; VEGF; EPO; TIMP-1) was performed on media collected 48 hours after the end of the preconditioning period. Cytokine levels were analyzed using Welch's t-Test. FSUs were fixed in 10% neutral-buffered formalin and decalcified in ImmunoCal for 72 hours. Samples were fixed in paraffin, sectioned in the sagittal plane at 10 μ m thickness, and stained with Safranin-O/Fast Green prior to being imaged via NanoZoomer. Histological images of the IVDs were evaluated for degeneration [10]. All procedures are WUSM IACUC-approved.

RESULTS: To contextualize changes in cytokine expression, previously presented data on upregulated db/db cytokines was graphed alongside upregulated STZHFD cytokines [5] (**Figure A**). A majority of the differentially upregulated chemokines are associated with proinflammatory immune cells, specifically M1 macrophages (CCL3, CCL12) and Type 1 helper (Th1) T cells (CCL5, CCL19, CXCL9) [11-20]. Other pleiotropic chemokines for general immune cell (IL-16) or neutrophil recruitment (CXCL2) are also elevated in the STZHFD IVDs [21,22]. Finally, CCL2 is responsible for recruiting monocytes and primarily promotes M2 macrophage polarization, although it has been shown to promote M1 macrophage polarization in certain contexts [23, 28]. This overwhelming shift in cytokine production may be responsible for contributing to the degeneration observed in STZHFD IVDs, a trend less prominent among db/db mice [7,24] (**Figure C**).

DISCUSSION: The potent increase in cytokine production evident among STZHFD mice affirms that T2D promotes a pro-inflammatory microenvironment that affects the IVD in a degenerative manner. Although these upregulated cytokines are associated with recruiting specific immune cell populations, whether the immune cells migrate into the IVD and their functional impact remain unknown. Future work could include single-cell RNAseq to discover the quantity and identity of potential infiltrating immune cells and immunofluorescent imaging to visualize these cells *in situ*. The differentially upregulated pro-inflammatory cytokines between db/db and STZHFD mice illustrate that leptin's established role as a pro-inflammatory adipokine role also applies to the IVD in obese T2D (**Figure B**) [6,7,24,25]. These findings indicate that future studies of T2D-associated inflammation should utilize leptin-preserving models of T2D, like the STZHFD model, to preserve the relevant physiology.

SIGNIFICANCE: These studies will identify key cytokines relevant to T2D-induced degeneration of the IVD. T2D-mediated inflammation may recruit immune cells that subsequently affect pain and degeneration [27].

REFERENCES: 1. Adams et al. 2006 2. Battié et al. 2006 3. Tuomi et al. 2005 4. Wyatt et al. 2006 5. Gonzalez et al. 2022 ORS Abstract No. 910 6. Francisco et al. 2018 7. Natelson et al. 2020 8. Furman et al. 2015 9. Bonora et al. 2011 10. Melgoza et al. 2021 11. Yuan et al. 2022 12. Xu et al. 2021 13. Zhang et al. 2016 14. DeLeon-Pennell et al. 2017 15. Shadidi et al. 2003 16. Sakthivel et al. 2008 17. Marsland et al. 2005 18. Westermann et al. 2007 19. Nguyen-Hoai et al. 2012 20. Koper et al. 2018 21. Glass et al. 2006 22. Al-Awan et al. 2013 23. Roca et al. 2009 24. Lintz et al. 2022 25. Segar et al. 2019 26. Sharma 2018 27. Risbud et al. 2014 28. Gschwandtner et al. 2019

ACKNOWLEDGEMENTS: This work was supported by the NIH R01AR074441 and P30 AR074992 g.

FIGURE A. STZHFD-induced T2D promotes differential upregulation of pro-inflammatory cytokines in the IVD when compared to those of db/db mice. **B.** While the db/db and STZHFD models share several upregulated cytokines, STZHFD-induced T2D exclusively promotes upregulation of many additional proinflammatory cytokines. **C.** STZHFD IVDs, especially the annulus fibrosus and cartilaginous endplate, have higher degeneration scores than control IVDs.

